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Morphological and Phylogenetic analysis of *Strobilomyces glabriceps* W.F. Chiu (*Boletaceae*) from Arunachal Pradesh, India

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ABSTRACT

During a mycofloristic survey and exploration of the Western Part of Arunachal Pradesh, India, several specimens belonging to the family Boletaceae were collected. Detailed morphological and phylogenetic analyses identified two of them as Strobilomyces glabriceps W.F. Chiu, a species previously reported from Western Himalaya only but unreported from the state of Arunachal Pradesh. This study provides a comprehensive description of the species, supported with morphological characteristics and phylogenetic relationships, first time from this region and also extends its range of occurrence in Eastern Himalayan Region.

Keywords: Arunachal Pradesh, *Boletaceae*, India, *Strobilomyces*, Taxonomy

INTRODUCTION

Strobilomyces Berk., is a distinct genus of the Family *Boletaceae* which is characterized by showing blackish, blackish-brown, reddish-brown or yellowish-brown, dark brown to blackish basidiomata with coarsely fibrillose or conical to patch-like scales on the pilear surface, context and hymenophore staining reddish to blackish when cut, and echinulate, reticulate to longitudinally striate basidiospores (Wu *et al.,* 2016, Deng *et al.,* 2023). The shaggy appearance of the basidiomata of this genus are characteristic and easy to be identified in the field. Presently there are 55 valid species exist in the genus *Strobilomyces* as per the Index Fungorum database [http://www.Indexfungorum.org (accessed on 25 June 2025), In India, at least 11 species (*S. annulatus* Corner, *S. echinocephalus* Gelardi & Vizzini, *S. glabriceps* W.F. Chiu, *S. indicus* Lloyd, *S. kalimpongensis* Bose, *S. longistipitatus* D. Chakr., K. Das & S. Adhikari, *S. mollis* Corner, *S. montosus* Berk., *S. nigricans* Berk., *S. polypyramis* Hook. f., *S. strobilaceus* (Scop.) Berk) have been documented from various parts of country (Murrill 1924, Bose 1946, Sharma & Lakhanpal 1981, Manjula 1983, Lakhanpal & Sharma 1988, Lakhanpal 1996, Kaur *et al.,* 2013, Tibpromma *et al.,* 2017, Han *et al.,* 2020,). *S. glabriceps* W.F. Chiu was originally described from Yunnan Province, China by W.F. Chiu (Chiu 1948). This species was not reported from Eastern Himalaya Region of India since its initial discovery. This article presents a detailed morphological description coupled with illustrations and phylogenetic estimation of this species. In the Present communication *Strobilomyces glabriceps* is recorded and described first time from the Eastern Himalayan Region of India.

MATERIAL & METHODS

Morphological studies

A routine survey was conducted in the western Arunachal Pradesh (West Kameng District) in the rainy season (July-August) of 2023, and specimens of various groups of Macro-fungi were collected. Before collection of specimens photographs of the specimens showing important macro morphological features were taken. Photographs of collected samples and their habitats were captured using Nikon P950 Camera and also with the mobile phone. Colour codes were provide after the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). After initial observations, the basidiomata were dried using artificial drier. Microscopic examination was performed using a light microscope (Olympus CX 41, and Olympus BX 52) on free-hand sections of the dried specimens. These sections were mounted in either lactophenol cotton blue, Melzer's reagent, or a mixture of 5% KOH, phloxine, and Congo red, either together or separately. Spore measurements were done from 30 randomly selected basidiospores. Spore size and shape (length/width ratio, Q) are presented as minimum-(mean)-maximum values. Herbarium names or as per the

Thiers (continuously updated). Field emission scanning electron microscopy (FESEM) was used to examine spore ornamentation. Dry spore prints were directly mounted on adhesive tape attached to a metallic stub, gold-coated, and scanned at various magnifications under high vacuum conditions. This analysis was conducted using Zeiss SEM Microscope installed at the BSI, ERC, Shillong, Meghalaya India. Specimens were deposited at Botanical Survey of India, Arunachal Pradesh Regional Centre Herbarium (ARUN), Itanagar (ARUN F 04 & ARUN F 05).

Phylogenetic analysis

Genomic DNA was extracted from 100 mg of dried mushroom samples with the NucleoSpin Plant II Kit (Macherey-Nagel) following the manufacturer's instructions. With the help of primers ITS1-F and ITS4; brpb2-6F and frpb2-7cR; ef1-983F and ef1-1567R PCR amplification was carried out for three nuclear loci, the internal transcribed spacer (ITS), the region between the conserved domains 6 and 7 of the second largest subunit of RNA polymerase II (rpb2) and the translation elongation factor 1-α (tef 1) respectively (White *et al.,* 1990, Gardes & Bruns 1993, Liu *et al.,* 1999, Moncalvo *et al.,* 2000, Matheny 2005, Rehner and Buckley 2005). The PCR amplification was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems). By using ExoSAP-IT (GE Healthcare) treatment PCR products were purified after amplification. For the Sequencing reaction a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol is used. Both strands of the PCR fragment were sequenced on a ABI 3500 DNA Analyzer (Applied Biosystems, USA) using the amplifying primers. Sequence Scanner Software ver. 1 (applied bio systems) was used for checking sequence quality. Sequence alignment, required editing and contig preparation of the obtained sequences were carried out using Geneious Pro ver. 5.1 (Drummond *et al.,* 2010). For the present study, six sequences (two each for ITS, rpb2 and tef 1-α) were generated from two separate collections of *S. glabriceps* (voucher no. AP 23-15 and AP 23-20) and subsequently deposited in GenBank (Table 1).

Phylogenetic analysis based on ITS, rpb2 and tef 1-α sequences data was carried out to establish the correct and accurate phylogenetic placement of our specimen. The ITS, rpb2 and tef 1-α sequences of the newly described species and closely allied sequences were obtained from BLASTn search from GenBank (www.ncbi.nlm.nih.gov/genbank) and recently published phylogenies (Han *et al.,* 2018, 2020; Deng *et al.,* 2023), from the Asia and World. Three separate raw datasets (ITS, rpb2 and tef 1-α) were created. All the datasets were aligned separately using the online version of the multiple sequence alignment program MAFFT ver. 7 (https://mafft.cbrc.jp/alignment/software) (Katoh *et al.,* 2019) with L-INS-i strategy. The alignments were checked and trimmed manually with the conserved motifs with MEGA ver. 7 (Kumar *et al.,* 2016). Species delimitation was first checked by single locus phylogenies. When significant conflict was not observed among the single locus phylogenies, single-locus datasets (ITS, rpb2 and tef 1-α) were concatenated into a multi-locus dataset using BioEdit ver. 7.0.9 (Hall 1999) and used it for the phylogenetic analyses. A total of 96 taxa were included in the analysis, with *Afroboletus sequestratus* L.H. Han, Buyck & Zhu L. Yang and *Afroboletus multijugus* Heinem. & Rammeloo are used as the outgroup. The combined dataset was phylogenetically analysed using maximum likelihood (ML) method. The ML was performed using raxmlGUI 2.0 (Edler *et al.,* 2021) with the GTRGAMMA substitution model. ML analysis was performed by applying the rapid boot strap algorithm with 1000 replicates were used to obtain nodal support values. The phylogenetic tree shows maximum likelihood bootstrap (MLbs) values ≥ 70% only. DNA sequences are deposited in NCBI GenBank (https://www. ncbi.nlm.nih.gov/ genbank/) with accession numbers- ITS:PV523902, 566929; rpb2: PV549298, 549299; tef1: 549300, 549301.

RESULTS

Phylogenetic Inferences:

The final combined dataset (nrITS + rpb2 + tef1) consists of 96 sequences, including our consensus sequences, encompassing 2055 characters including gaps. The combined 3-locus phylogenetic analyses show that sequences derived from our Indian collections nested (with robust support of MLbs = 98%) within the clade consisting of three *S. glabriceps* collections from China and India. Our phylogenetic analyses strongly suggest that the present specimen is conspecific with other Asian collections of *S. glabriceps.*

TAXONOMIC DESCRIPTION

Strobilomyces glabriceps W.F. Chiu, *Mycologia* **40**(2): 229 (1948) (Fig. 1 & 2)

Figure 1: Phylogram generated by maximum likelihood (ML) analysis based on ITS, Rpb2 and Tef1 sequences data for *Strobilomyces glabriceps* and allied species. Maximum likelihood bootstrap support values (MLbs) are shown near the branches. This new record to Eastern Himalaya is highlighted in red to mark their phylogenetic positions in the tree.

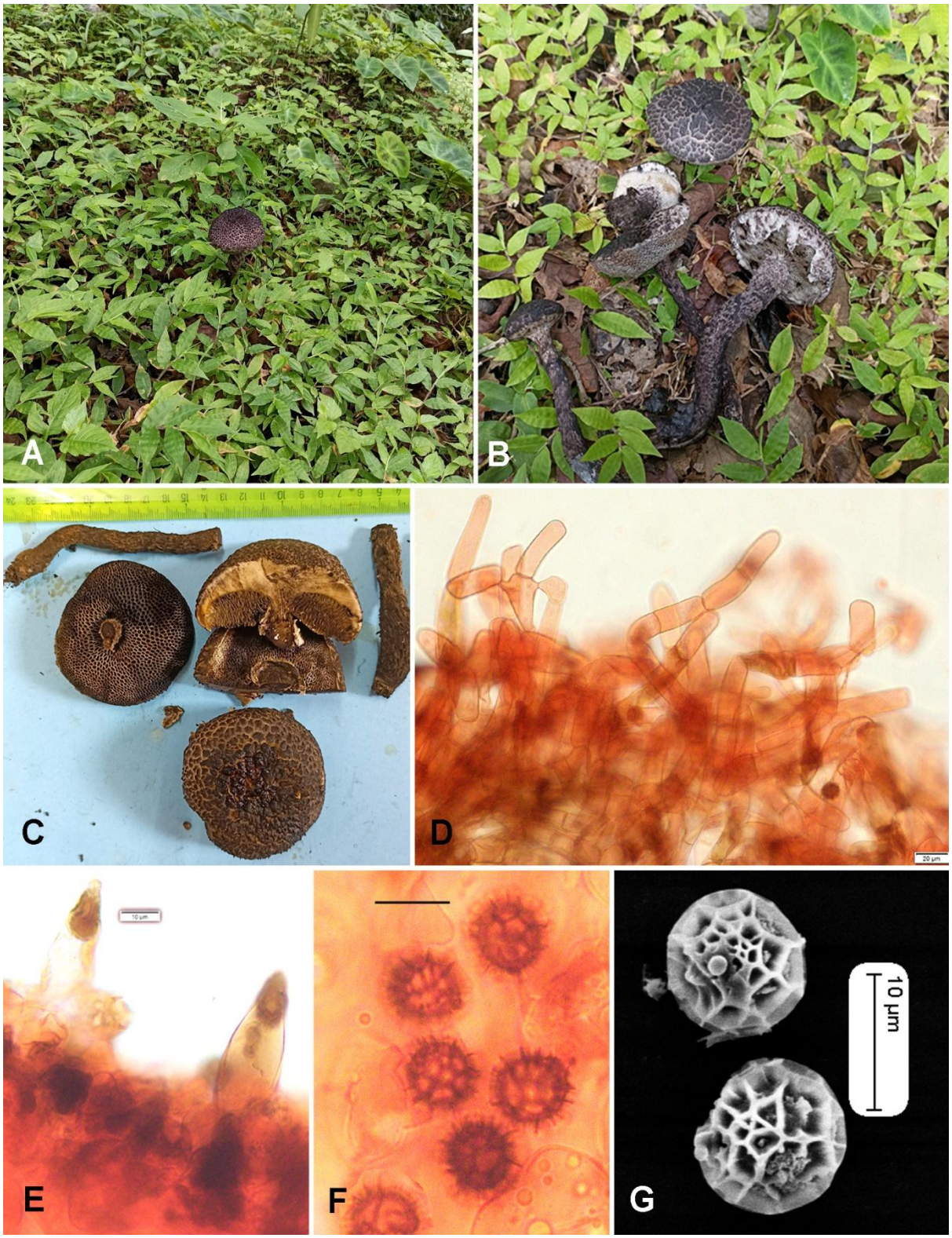

Figure 2: *Strobilomyces glabriceps*. A, Habit of the basidiomata; B, Basidiomata showing upper surface and pore surface; C, Basidiomata with scale showing upper surface pore surface and context; D, Terminal cells of Pileipellis; E, Pleurocystidia; F, Basidiospores; G, SEM Images of Basidiospores. Scale bars: D = 20 µm; E–G = 10 µm

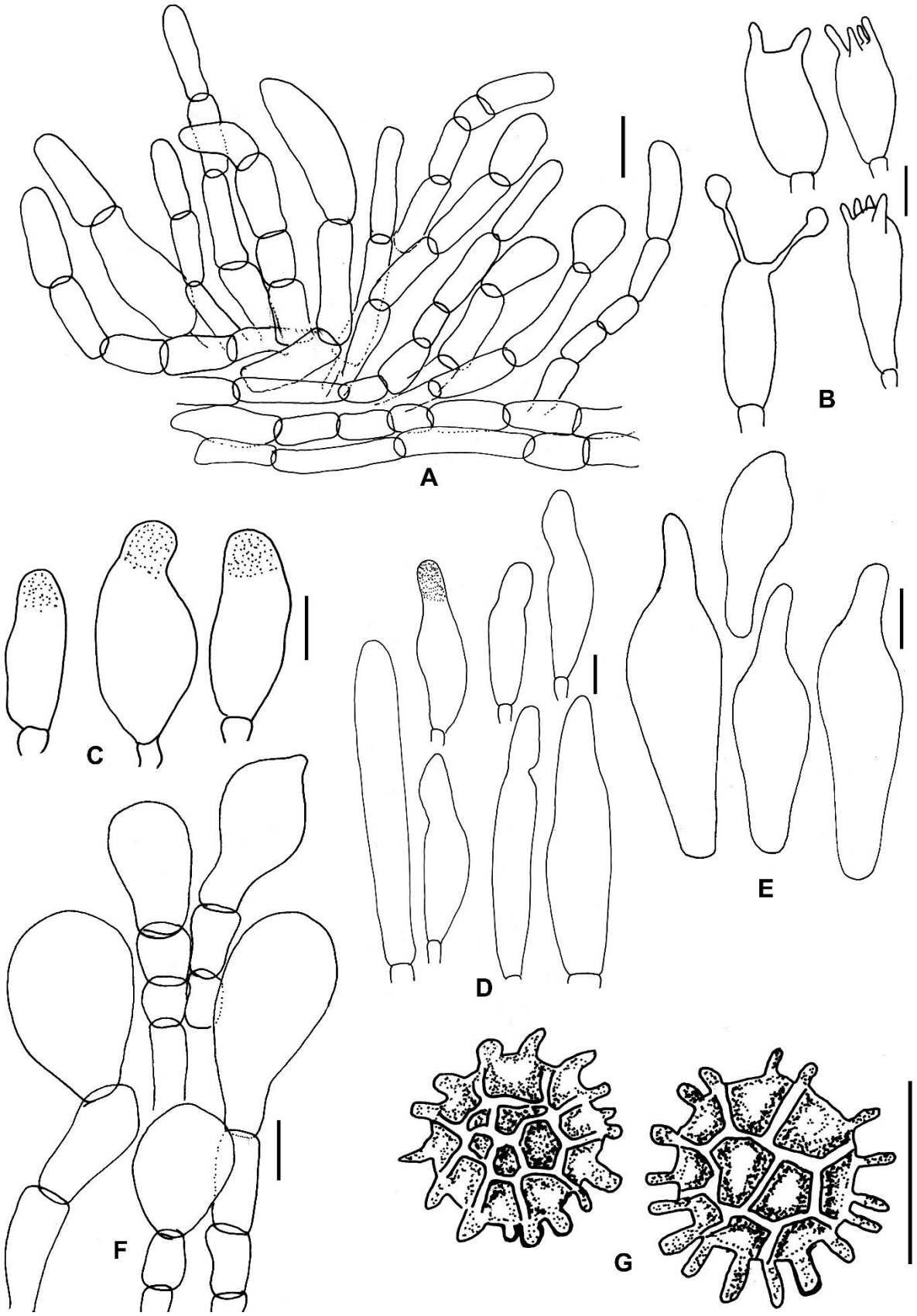

Figure 3: *Strobilomyces glabriceps*. A, Pileipellis; B, Basidia; C, Cheilocystidia; D, Pleurocystidia; E, Caulocystidia from stipe; F, Terminal cells of Stipitipellis; G, Basidiospores. Scale bars: A = 50 µm; B–G = 10 µm

Pileus 45–90 mm diameter, subhemispherical when young, then convex to applanate; surface brownish orange (6C3), densely covered with grey (11E1–11F1) to almost charcoal balck, patch-like to appressed scales; margin usually appendiculate with thick and triangular or irregular lacy veil remnants concolorous with pileal surface; context 8–12 mm in thickness in the center of the pileus, redsdish grey (8B2–9B2) after some time of exposure, greyish orange (5B4–5B5) with KOH. Hymenophore poroid, slightly depressed near the apex of the stipe; pores, 1–2 per mm, angular, tubes 10–12 mm long, brownish grey (8D2–9D2), turning dark brown (10F1) to almost black when injured, reddish brown (8D8–8E8) with KOH. Stipe 90–140 mm long 9–14 mm thick, central, subcylindrical, solid, grey (8E1–9E1); surface covered with patch-like to appressed blackish-brown (7E1) scales, reticulate near the stipe; context brownish grey (10E2–10 F2), turning reddish-brown (8E4) then dark brown (8F4); annulus present in the young stage of basidiomata, cottony grey (8B2–9B2) , .

Basidia 19–35 × 8–15 µm, clavate to subclavate, thin to thick-walled (up to 0.5 m), hyaline, yellowish-brown in KOH; sterigmata 2–8 µm in length. Basidiospores 8.5–(9.15)–11 × 8–(8.98)–10.5 µm, Q = 1.00–(1.07)–1.2 excluding ornamentation, globose to sub globose yellowish-brown to dark-brown in KOH, reticulate, with meshes 1–2 µm high. Hymenophoral trama boletoid, composed of hyaline to pale yellow hyphae, 9–12 µm wide, thin- to slightly thick-walled (up to 0.5 µm); Cheilocystidia 37–55 × 13–20 µm, fusiform or subfusiform, thin- to slightly thick-walled (up to 1 µm), colorless to pale yellowish-brown or yellowish-brown in KOH, slightly incrusted at the apex; Pleurocystidia 29–85 × 8–14 µm, fusiform to subfusiform, thin- to slightly thick-walled (up to 0.5 µm), yellowish-brown in KOH. Pileipellis a trichodermium 260–350 µm thick, terminal cells 39–55 × 11–17 µm, cylindrical, thin-walled with brown pigment. Stipitipellis a trichoderma-like structure 100–120 µm thick, composed of thin-walled pale yellowish-brown to yellowish-brown hyphae with clavate or subglobose terminal cells (12–16 × 7–11 µm). Stipe trama composed of parallel hyphae, tramal hyphae 4–12 µm wide, cylindrical, thin- to thick-walled (up to 0.5 µm), hyaline or pale yellowish-brown in KOH. Pileal trama 100–120 µm wide, tramal hyphae 9–12 µm wide, thin- to thick-walled (up to 0.5 µm), yellow in KOH. Clamp connections absent in all hyphae.

Habitat: Scattered on the ground in forests dominated by trees of Fagaceae especially Quercus sp.

Known distribution: China, Japan and India.

Specimen Examined: INDIA, Arunachal Pradesh, West Kameng District, Dirang, Yong Basti, Alt. 2068 m, N 27° 22′ 15.53″, E 92° 13′ 23.88″ 12 August 2023, Arvind Parihar AP 23-15 (ARUN F 04). Ibid., Yong basti Alt. 2063 m, N 27° 22′ 15.83″, E 92° 13′ 24.09″ 12 August 2023, Arvind Parihar AP 23-20 (ARUN F 05).

GenBank Accession Numbers: ITS GenBank No: PV523902, PV566929; RPB2 GenBank No: PV549298, PV549299; TEF1 GenBank No. PV549300, PV549301.

DISCUSSION

Identification of the genus *Strobilomyces* is relatively easy, and it can be separated from other boletoid species by shaggy appearance of its basidiomata which are greyish brown to blackish throughout, with conspicuous reddening or blackening of fresh tissues when bruised or exposed (Gelardi *et al.,* 2012). However, species-level identification within this genus remains challenging due to limited molecular data and morphological similarities among species. Species delimitation of *Strobilomyces* has mainly depended on macromorphological and ecological characters of various species, i.e., the size and shape of the pileus, colour, size and morphology of the scales on the pileus and stipe surface, size of pores and tubes, colour changing of the exposed context, presence or absence of an annulus or an annular zone, association with host plants and geographical distributions (Corner 1972, Ying & Ma 1985; Zeng 1985, Singer 1986, Sato *et al.*, 2011, Han *et al.,* 2018). The present species is unique in having macroscopic characters like medium size of pileus (up to 90 mm), patch like light coloured scales on the pilear surface, stipe with a slightly bulbous base, grey-black discolouration of the context on exposure, and specific microscopic features like globose to subglobose basidiospores. The size of basidiomata and basidiospores are within the range of its original description (Chiu 1948). Morphologically, this species is similar to *S. douformis* L.H. Han & Zhu L. Yang, but presence of black scales on pileus, absence of annulus, abundant chielocystidia separate it from

the present species (Han *et al.,* 2020). Phylogenetically this species is close to *S. pteroreticulosporus*, but it can be separated by its slightly larger, light coloured basidiomata with smaller erect conical scales (1–3 mm high, 1–3 mm diam at base) on the pileus, rusty red discolouration on exposure, and preferable association with Pinus spp, and a peculiar spicy smell. Microscopically, slightly larger basidiospores (9.5–12× 9–10.5) with distinct high reticulate ornamentation and rare and large size of pleurocystidia (37–70 × 15–26) are the distinct features for *S. pteroreticulosporus* (Antonin *et al.,* 2015).

CONCLUSIONS

This first report of *Strobilomyces glabriceps* from the Arunachal Pradesh is very significant and it shows the potential of diversity of Boletes and specifically the Genus *Strobilomyces* in the state of Arunachal Pradesh. Still many species of this unique group are yet to be discovered and recorded from the forests of Arunachal Pradesh, India. However the correct identification and proper taxonomic placement of the species within the genus *Strobilomyces* is difficult and challenging and it required both Morphological and molecular approach. Additionally many areas of the Arunachal Pradesh are still not surveyed in terms of macrofungal survey and also not easily approachable. A thorough survey in these areas could result into many more such discoveries.

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DECLARATION

Author declare no conflict of interest.

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